ROS Technology, Test to Evaluate Ability of Air Disinfection Biosecurity (ADB) Technology to Eliminate *Influenza A virus (H1N1).*

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ABSTRACT

Influenzavirus A is a genus of the Orthomyxoviridae family of viruses. Influenzavirus A includes only one species: *Influenza A virus* which causes influenza in birds and some mammals. Strains of all subtypes of *influenza A* virus have been isolated from wild birds, although disease is uncommon. Some isolates of *influenza A* virus cause severe disease both in domestic poultry and, rarely, in humans. Occasionally viruses are transmitted from wild aquatic birds to domestic poultry and this may cause an outbreak or give rise to human influenza pandemics.

Variants are identified and named according to the isolate that they are like and thus are presumed to share lineage (example Fujian flu virus like); according to their typical host (example Human flu virus); according to their subtype (example H3N2); and according to their deadliness (example LP). So a flu from a virus similar to the isolate A/Fujian/411/2002(H3N2) is called Fujian flu, human flu, and H3N2 flu.

Variants are sometimes named according to the species (host) the strain is endemic in or adapted to. The main variants named using this convention are: Bird flu, Human flu, Swine flu, Horse flu, Dog flu have also sometimes been named according to their deadliness in poultry, especially chickens:

- Low Pathogenic Avian Influenza (LPAI)
- Highly Pathogenic Avian Influenza (HPAI), also called: deadly flu or death flu

The Influenza A virus subtypes are labeled according to an H number (for the type of hemagglutinin) and an N number (for the type of neuraminidase). Each subtype virus has mutated into a variety of strains with differing pathogenic profiles; some pathogenic to one species but not others, some pathogenic to multiple species. Most known strains are extinct strains. For example, the annual flu subtype H3N2 no longer contains the strain that caused the Hong Kong Flu.

Influenza A viruses are negative sense, single-stranded, segmented RNA viruses. There are 16 different HA antigens (H1 to H16) and nine different NA antigens (N1 to N9) for influenza A. Until recently, 15 HA types had been recognized, but a new type (H16) was isolated from black-headed gulls caught in Sweden and the Netherlands in 1999 and reported in the literature in 2005.

MATERIALS AND METHODS

Orthomyxoviridae, Influenza A virus (H1N1), ATCC # VR-897, was acquired from ATCC, Manassas, VA., USA and propagated in 10 day embryonated hen eggs (University California Davis Poultry Science, Davis, CA) to approximately 5.0-$\log_{10}$ $TCID_{50}$ (as determined in Madin Darby Canine Kidney, MDCK cells). Cells were maintained in Minimal Essential Medium with Earle's salts and L-glutamine (Invitrogen Corporation, Carlsbad, CA) and 2.2 g/L sodium bicarbonate (Fisher Scientific, Pittsburgh, PA) collectively referred to as MEM containing 10% fetal bovine serum (FBS, Fisher

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BioReagents, Fair Lawn, NJ) supplemented with antibiotics [2.5 mg/L amphotericin B; 0.67 g/L streptomycin; and 0.3 g/L penicillin G (all from Fisher Scientific)]. Infectivity media was made by adding MEM with the addition of 0.1% TPCK treated trypsin (Fisher Scientific) and supplemented with antibiotics (2.5 mg/L amphotericin B; 0.67 g/L streptomycin; and 0.3 g/L penicillin G).

H1N1 Inactivation

Approximately 5 ml of egg propagated H1N1 was atomized (26 micron droplets) via model CF40K50T atomizer (Sonaer®, Farmingdale, NY). The atomized inoculum was introduced to the suction side of the Catalyst Reactor Unit near the intake aperture of the unit, the fan speed was set to **full** and array voltage set at **40**. Infectivity media, 20 ml was poured in each sterilized disposable plastic petri dishes 100 x 15 mm (Fisher Scientific) which were placed at the discharge end of the Catalyst Reactor Unit, as "catch plates". The array of "catch plates" were arranged so four plates, two close (approximately 3 inches) and two further away (approximately 6 inches) could be removed at 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 minute intervals.

Virus Recovery

H1N1 virus was recovered from "catch plates" by pouring the contents (approximately 20 ml infectivity media) from the individual "catch plate" to a sterile 50 ml conical vial (Fisher Scientific). Tubes were then vortexed for 1 min. Endpoint dilution titration was conducted in MDCK cells by adding 220 µl from the 20 ml infectivity media containing any suspended virus to the first dilution well in a minimum of 6 wells of a 96 well microtiter plate containing confluent MDCK cells. Then, serial 1:10 dilutions were prepared by adding 20 µl from the first well into the next 6 wells each containing 180 µl infectivity media. The final well contained only 200 µl infectivity media to serve as a negative cellular control. Plates were incubated at 37°C, 5% CO2 for 48 hours. Cytopathic effect (CPE) was determined for each well and viral counts were reported as TCID50/ml as calculated by Reed and Muench (6).

Real-Time Reverse Transcription Polymerase Chain Reaction *(r*RT-PCR*).*

Viral RNA was recovered using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). Quantitative detection of the extracted influenza RNA was conducted using *r*RT-PCR using a fluorescently labeled TaqMan-MGB (minor groove binder) probe. The *r*RT-PCR primer and probe sequences were provided by Molecular Genetics Influenza Branch, Centers for Disease Control and Prevention in Atlanta, GA. The detection threshold for successfully detecting influenza RNA was a FAM fluorescence signal ≥ 3 using the SmartCycler.

RESULTS

The average amount of H1N1 recovered from the 0.0 "catch plates" in this experiment was 5.0 $\log_{10}$ $TCID_{50}$/ml. Following treatment with the Reactive Oxygen Species produced by the Catalyst Reactor Unit, the average log reductions of the H1N1 virus was 2.7-$\log_{10}$ and 5.0-$\log_{10}$ $TCID_{50}$/ml following 30 second and 1 minute treatments, respectively, based on the infectious virus recovery. The average amount of viral H1N1 RNA recovered from the traveling control in all experiments was 5.0-$\log_{10}$ based on a quantitative RT-PCR for influenza A virus (Table 1).

Due to the rapid inactivation of the H1N1 is it suggested that the mechanism of action for loss of infectivity was more likely due to disruption of the lipid envelope or structural proteins than with

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degradation of the viral nucleic acid. This can be substantiated in subsequent experiments via quantitative RT-PCR.

Table 1

Recovery of H1N1 post-treatment with ROS produced by the ADB unit based on TCID50/ml in MDCK cells.

Time Minutes	Concentration Log $TCID_{50}$/ml	Avg. Destruction Log	RH %	Temperature °F	O3 ppm	Hydrogen Peroxide ppm
0.0	5.0	-	29	78	0.001	0.02
0.5	2.3	2.7	29	79	0.001	0.02
1.0	0.0	5.0	29	79	0.001	0.02
1.5	0.0	5.0	29	78	0.001	0.02
2.0	0.0	5.0	29	78	0.001	0.02
2.5	0.0	5.0	28	78	0.001	0.02
3.0	0.0	5.0	28	78	0.001	0.02

DISCUSSION

Current International Committee on Taxonomy of Viruses Index of Viruses guidelines is to achieve a > 4.0-log_{10} reduction in a starting virus titer (1). ROS treatment for 1 minute resulted in the successful inactivation of the H1N1 isolate from a starting contamination level of 5.0 log_{10} $TCID_{50}$/ml.

Since the inoculum was atomized near but not directly into the ADB unit some over-spray occurred and was destroyed as soon as it passed through the unit.

As for contamination levels lower than 5.0 log_{10} $TCID_{50}$/ml, which might be more representative in a natural environmental outbreak, additional testing would be required to determine if lower exposure times would result in complete inactivation (5, 8, 10).

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Regards,

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